Bacterial Transformation - *pGlo*

Introduction:

Introduction to Transformation - Genetic transformation is taking genes from one organism and putting them in another. A gene is a piece of DNA that gives the instructions for making a protein. This protein gives an organism a certain trait. A gene is inserted into an organism in order to change the organism's trait. Genetic transformation is used in many areas of biotechnology. In agriculture, genes coding for traits such as frost, pest, or spoilage resistance can be inserted into plants. In medicine, gene therapy treats diseases caused by defective genes by inserting healthy copies of the defective gene in a sick person's cells.

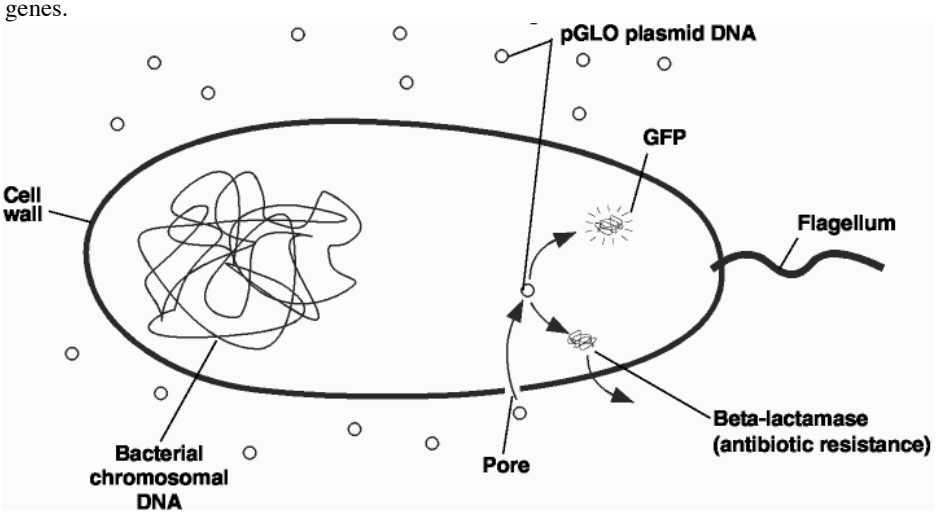
You will transform bacteria with a gene that codes for Green Fluorescent Protein (GFP). The real-life source of this gene is a jellyfish. GFP causes certain areas of the jellyfish to glow in the dark. After you transform the bacteria, they will express their new jellyfish gene and produce the fluorescent protein. It causes them to glow a brilliant green color under ultraviolet light.

You will learn about the process of moving genes from one organism to another with the aid of a plasmid, a small circular piece of DNA. Bacteria have one large chromosome and one or more plasmids. Plasmids usually contain genes for traits that may help the bacteria survive. In nature, bacteria can transfer plasmids back and forth allowing them to share these beneficial genes. This process allows bacteria to adapt to new environments.

Bio-Rad's pGLO plasmid has three special genes: one for GFP, a gene for antibiotic resistance, and a gene regulation system. This system can be used to control when the bacteria produce fluorescent protein. The gene for GFP can be switched on in transformed cells by adding the sugar arabinose to the cells' food source. Transformed cells will appear white on plates not containing arabinose, and fluorescent green when arabinose is included in the nutrient agar medium. Also, we can test that cells have been transformed with pGLO DNA by growing them on antibiotic plates.

The Genes - Genetic transformation involves the insertion of some new DNA into the E. coli cells. Bacteria have one large chromosome and one or more plasmids. Plasmids usually contain genes for traits that may help the bacteria survive. Scientists can use a process called genetic engineering to insert genes coding for new traits into a plasmid. In this lab, the pGLO plasmid has the GFP gene that codes for the green fluorescent protein and a gene that codes for a protein that gives the bacteria resistance to an antibiotic. The pGlo plasmid can then be used to transform bacteria to give them this new trait.

The Act of Transformation - This transformation procedure involves three main steps. These steps are intended to insert the plasmid DNA into the E. coli cells and provide an environment for the cells to produce their newly acquired genes.



A. To move the pGLO plasmid DNA through the cell membrane you will:

1.) Use a **transformation solution** of $CaCl_2$ (calcium chloride) to make cells **competent**

Competent Cells - Bacterial cells must be in a particular state before they can be transformed. This state is referred to as *competency*. This state can be achieved naturally in some species of bacteria when levels of nutrients and oxygen are low. E.coli, the organism on which most current research is performed, must be artificially induced to make it competent. Competent E. coli cells are very fragile and must be treated carefully.

Transformation Solution: Calcium Chloride $CaCl_2$ - The bacterial cell membrane is permeable to chloride ions, but is non-permeable to calcium ions. As the chloride ions enter the cell, water molecules accompany the charged particle. This influx of water causes the cells to swell and is necessary for the uptake of DNA. The exact mechanism of this uptake is unknown. It is known, however, that the calcium chloride treatment should be followed by heat.

2.) Carry out a procedure referred to as **heat shock** so bacteria can take in the plasmid

Heat Shock Treatment - When E.coli are subjected to 42°C heat, a set of genes are expressed which aid the bacteria in surviving at that temperature. This set of genes is called the heat shock genes. The heat shock step is necessary for the uptake of DNA. At temperatures above 42°C, the bacteria's ability to uptake DNA is lowered, and at extreme temperatures the bacteria will die.

B. For transformed cells to grow in the presence of ampicillin you must:

3.) Provide nutrients and a short **incubation period** to begin expressing the newly acquired genes

Incubation - After the heat shock step, intact plasmid DNA molecules replicate in bacterial host cells. To help the bacterial cells recover from the heat shock, the cells are briefly incubated in LB Nutrient Broth, a solution that provides nutrients for the bacteria. As the cells recover, plasmid genes are expressed, including those that allow the replication of plasmids which will end up in new, dividing bacterial cells.

Genetic Regulation - Our bodies contain thousands of different proteins which perform many different jobs. Digestive enzymes are proteins; some of the hormone signals that run through our bodies and the antibodies protecting us from disease are proteins. The information for assembling a protein is carried in our DNA. The section of DNA which contains the code for making a protein is called a gene. There are over 30,000–100,000 genes in the human genome. Each gene codes for a unique protein: one gene, one protein. The gene that codes for a digestive enzyme in your mouth is different from one that codes for an antibody or the pigment that colors your eyes.

Organisms regulate expression of their genes and ultimately the amounts and kinds of proteins present within their cells for many reasons, including developmental changes, cellular specialization, and adaptation to the environment. Gene regulation not only allows for adaptation to differing conditions, but also prevents wasteful overproduction of unneeded proteins which would put the organism at a competitive disadvantage. The genes involved in the transport and breakdown (catabolism) of food are good examples of highly regulated genes. For example, the sugar arabinose is both a source of energy and a source of carbon. *E. coli* bacteria produce three enzymes (proteins) needed to digest arabinose as a food source. The genes which code for these enzymes are not expressed when arabinose is absent, but they are expressed when arabinose is present in their environment. How is this so?

Regulation of the expression of proteins often occurs at the level of transcription from DNA into RNA. This regulation takes place at a very specific location on the DNA template, called a promoter, where RNA polymerase sits down on the DNA and begins transcription of the gene. In bacteria, groups of related genes are often clustered together and transcribed into RNA from one promoter. These clusters of genes controlled by a single promoter are called operons. The three genes *(araB, araA* and *araD)* that code for three digestive enzymes involved in the breakdown of arabinose are clustered together in what is known as the arabinose operon. When arabinose is present in the environment, bacteria take it up. Once inside, the arabinose interacts directly with arabinose operon and the interaction causes the transcription of the three digestive enzyme genes. When the three enzymes are produced, they break down arabinose, and eventually the arabinose runs out. In the absence of arabinose the transcription is shut off.

The DNA code of the pGLO plasmid has been engineered to incorporate aspects of the arabinose operon. The genes which code for break down of arabinose, *araB, A* and *D,* have been replaced by the single gene which codes for GFP. Therefore, in the presence of arabinose, GFP is produced. Cells fluoresce brilliant green as they produce more and more GFP. In the absence of arabinose, GFP gene is not transcribed. When GFP is not made, bacteria colonies will appear to have a wild-type (natural) phenotype—of white colonies with no fluorescence.

Genetic Selection - Not all cells will have the plasmid and not all newly produced plasmids will end up in new bacteria cells. So, it is necessary to select for bacterial cells which contain the plasmid. This is commonly performed with antibiotic selection. Some E.coli strains cannot grow in the presence of common antibiotics like ampicillin. Plasmids used for the cloning and manipulation of DNA have been engineered to contain the genes for antibiotic resistance. Thus, if the bacterial transformation is plated onto media containing ampicillin, only bacteria which have the plasmid DNA will have the ability to metabolize ampicillin and form colonies. In this way, bacterial cells containing plasmid DNA are selected.

Materials and Methods:

PART I - Pre-Lab Observations: The goal of genetic transformation is to change an organism's traits (phenotype). Before any change in the phenotype of an organism can be seen, a good examination of its natural phenotype must be made. Look at the colonies of E. coli on your starter plates. The following pre-lab observations of E. coli will provide basic data to make reference to when attempting to determine if any genetic transformation has occurred.

Use the following traits in table 1 to describe what you see: ***Use a separate sheet of paper to record your observations. Include your name, group number, and the headings below on the paper***

Colony Information	Recorded Measurement
Total Number of Colonies	
Size of Colonies (Largest, Smallest, Average) in mm	
Color of Colonies	
Distribution of Colonies on the Plate	
Visible Appearance of Colonies viewed w/ UV Light	

Table 1

PART II - pGlo Bacterial Transformation Procedure

1. At your group's lab station, check to make sure you have the following materials:
   - □ Test tube labeled, **+pGlo**, containing **250 µl** of **transformation solution** ($CaC1_2$)
   - □ Test tube labeled, **-pGlo**, containing **250 µl** of **transformation solution** ($CaC1_2$)
   - □ Test tube labeled, **LB,** containing 1ml of **LB Nutrient Broth**
     - *The cap of each tube should have your group's number written on it.
     - *The tubes should be in a foam rack on ice.
   - □ One E.coli starter plate (with colonies of E.coli growing on it)
   - □ 4 genetic selection plates (1 LB, 2 LB/Amp, 1 LB/Amp/Ara)
   - □ 1 packet of sterile loops
   - □ 6 sterile pipets in individual packages

In order to minimize contamination from exposure to the air, ensure all tubes and plates are closed when not in use. When tubes and plates are in use, make sure they are only open for a short time. For plates, only lift the lids enough to insert loops or pipets. Do not completely remove lids and place on the counter-top. This will expose the entire plate to possible contamination.

2. Use a sterile loop to pick up a single colony of bacteria from your E.coli starter plate.
In order to avoid contamination, only remove the loop from the package right before you use it and do not touch the loop to any other surfaces besides the starter plate.

3. Pick up the **+pGLO tube** and place the loop into the transformation solution at the bottom of the tube. Spin the loop between your index finger and thumb until the entire colony is dispersed in the transformation solution (with no floating chunks). Place the tube back in the tube rack in the ice.

4. Using a **new sterile loop**, repeat steps 2 and 3 for the **-pGLO tube**.

5. Take your **+pGLO tube** to your instructor to receive the pGlo plasmid. Immerse a **new sterile loop** into the **plasmid DNA stock tube**. Withdraw a loopful. *The loop should have a film on it, as if you were blowing bubbles.* Mix the loopful into the cell suspension of the **+pGLO tube**. Close the tube and return it to the rack on ice. Close the **-pGLO** tube. **Do not add plasmid DNA to the -pGLO tube.**

6. Incubate the tubes on ice for 10 minutes. Push the tubes all the way down in the rack so the bottoms of the tubes make contact with the ice.

7. While the tubes are sitting on ice, label your four agar plates **on the bottom**. Follow the picture below. *Label your tubes as close to the edge as possible so that you can view your results.*

8. **Heat shock.** Using the foam rack as a holder, transfer both the (+) pGLO and (-) pGLO tubes into the water bath, set at 42 °C, **for exactly 50 seconds**.

9. When the 50 seconds are done, incubate tubes on ice for **2 minutes**.

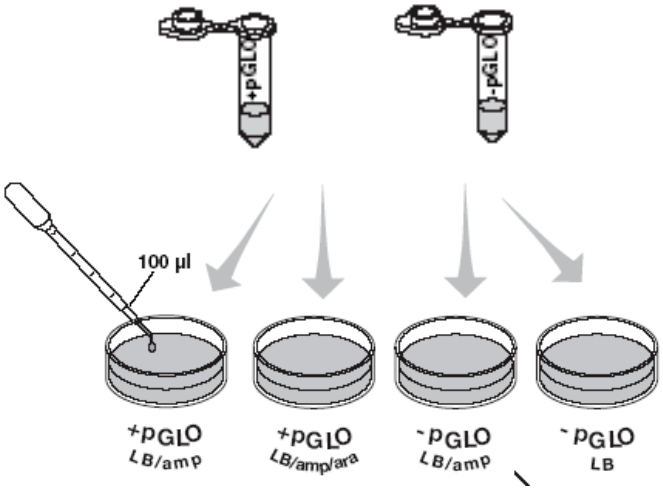
10. Place the foam tube rack on the bench top. Open a new sterile pipet and add **250 µl of LB nutrient broth** to the **+pGLO tube**. ***250 µl is the 3rd section up on the pipet.***
In order to avoid contamination, open the pipet package from the top (near the bulb) and do not touch the pipet tip to any other surfaces.

11. Repeat step 14 with a new sterile pipet for the **-pGLO tube**.

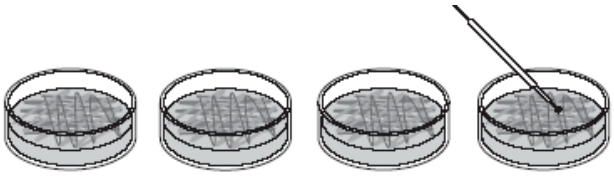
12. Incubate the tubes for 10 minutes at room temperature.

13. After the 10 minutes incubation, tap the closed tubes with your finger to mix.

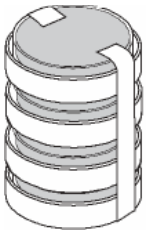
14. Using a new sterile pipet for each tube, **pipet 100 µl of the transformation and control** onto the correct plates. ***100 µl is the second section up on the pipet.*** *Make sure to pipet the suspensions onto the agar surface of the plates, NOT onto the lids of the plates*



15. Use a new sterile loop for each plate. Spread the suspensions evenly around the surface of the agar by skimming the flat surface of the loop back and forth across the surface. *DO NOT dig the loops into the surface of the agar and create marks on the surface*.



16. Stack up your plates and tape them together. Put your group number and class period on the bottom of the stack and place the stack upside down in the 37°C incubator until the next day. Discard all tubes in your waste beaker. Leave your E.coli starter plate at your station. *Wash your hands thoroughly before leaving class.*



<u>Results</u>
Before collecting data and analyzing your results, answer the following questions.

1. Which plate do you expect to find bacteria most like the original E.coli colonies you observed?
   a. +pGlo, LB/amp Explain your prediction:
   b. +pGlo, LB/amp/ara
   c. –pGlo, LB/amp
   d. –pGlo, LB

2. On which plate or plates would you expect to find genetically transformed bacteria?
   a. +pGlo, LB/amp Explain your prediction:
   b. +pGlo, LB/amp/ara
   c. –pGlo, LB/amp
   d. –pGlo, LB

3. Which plates should be compared to determine if any genetic transformation has occurred?
   a. +pGlo, LB/amp Why?
   b. +pGlo, LB/amp/ara
   c. –pGlo, LB/amp
   d. –pGlo, LB

4. Which plate or plates would be considered control plates in this lab?

a. +pGlo, LB/amp Why?
b. +pGlo, LB/amp/ara
c. –pGlo, LB/amp
d. –pGlo, LB

5. At the time you spread your transformed bacterial cells on the LB/amp/ara plate, how many individual cells were on the plate? Were the cells visible?

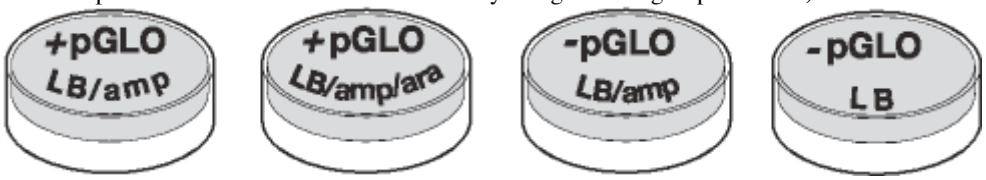
If the number of colonies you end up with is the same as the number of cells originally on the plate, how many cells are there in each colony after 24 hours of growth? The following equation shows the number of cells per colony.

Colonies are grown for 24 hours and there are 60 minutes/hour. E.coli bacteria double every 40 minutes. This means: Colonies = (24 hrs) X (60 min/hr) X (1 doubling/40 min) = 36 doublings

Number of cells per colony (starting with one cell) = 236 = 6.8 x 10^{10}

There are almost 70 billion cells per colony!!!

6. Review your predictions from the Pre-Lab worksheet for bacterial growth. What are your actual results? Under each plate below, list your observations. (If you did not end up with any glowing bacteria, indicate this in your observations, by placing an "X" over the plates below. Fill in the observations by using another group's results.)



Pattern of growth: (no growth, single colonies, or lawn)	_____	_____	_____	_____
Average size of colonies (mm):	_____	_____	_____	_____
Color of colonies under *normal lighting*:	_____	_____	_____	_____
Color of colonies under *UV light*:	_____	_____	_____	_____
Number of colonies:	_____	_____	_____	_____
Additional Notes:	_____	_____	_____	_____

2. Which traits that you initially observed for E.coli **did not** change?

3. Which traits that you initially observed for E.coli **did** change after performing the procedure?